

AS 48. (New) The method according to claim 27 wherein said cells express beta lactamase at high levels.

REMARKS

In the Specification:

Amendments to paragraph numbers 0027, 0053, 0098, 0147, 0289, 0298, 0307, 0314, 0321, 0360, 0373, 0378, 0379, and 0383 were made to correct typographical errors or to provide clarity to terms well known to one skilled in the art (for example, addition of "SWISS-PROT" to each accession number citation in paragraph 0098 to clarify the database to which each Accession number refers). No new matter has been added. Marked copies of each paragraph delineating each amendment are submitted herewith.

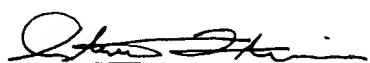
In the Claims:

Claims 1- 22 have been cancelled and new claims 23-48 have been added. Support for the newly added claims may be found in the application as originally filed. Specifically, support for new claims 23-29 may be found in paragraph numbers 0018, 0113, 0284-0298, 0157, 231-236, 0251-0252, original claims 10, 12, 17, 18, 19, 20, 21, 22, and throughout the application as originally filed. Support for new claims 30-33, and 45-48 may be found in paragraph numbers 0051, 0298, Figure 16A-D, and throughout the application as originally filed. Support for new claim 34 and 37 may be found in paragraph numbers 0251, 0258, 0262-0263, and throughout the application as originally filed. Support for new claim 35 and 38 may be found in paragraph numbers 0024, 0128, 0318-0319, and throughout the application as originally filed. Support for new claim 36 and 39 may be found in paragraph numbers 0018, 0067, 0224, and throughout the application as originally filed. Support for new claim 40-43 may be found in paragraph numbers 0011, 0021, 0051, and throughout the application as originally filed. Support for new claim 44 may be found in paragraph numbers 0026, 0096, 0155-0157, and throughout the application as originally filed. No new matter has been added. Applicants believe that all of the pending claims before the Examiner are in condition for allowance. An early Office Action to that effect is, therefore, earnestly solicited.

If any fee is due in connection herewith not already accounted for, please charge such fee to Deposit Account No. 19-3880 of the undersigned. Furthermore, if any extension of time not already accounted for is required, such extension is hereby petitioned for, and it is requested that any fee due for said extension be charged to the above-stated Deposit Account.

Respectfully submitted,

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Date: August 8th, 2002



VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

On page 6, replace paragraph [0027] with the following paragraph:

[0027] It is an object of the present invention to also provide a method for detecting a polynucleotide that encodes a G-protein coupled receptor, preferably the HGPRBMY8 polypeptide, or homologue, or fragment thereof, in a biological sample comprising the steps of: a) hybridizing the polynucleotide, or complement of the polynucleotide sequence encoding SEQ ID NO:2 to a nucleic acid material of a biological sample, thereby forming a hybridization complex; and b) detecting the hybridization complex, wherein the presence of the complex correlates with the presence of a polynucleotide encoding the HGPRBMY8 polypeptide, or fragment thereof, in the biological sample. The nucleic acid material may be further amplified by the polymerase chain reaction prior to hybridization.

On page 10, replace paragraph [0053] with the following paragraph:

[0053] Figures 18A-B show the polynucleotide sequence (SEQ ID NO:48) and deduced amino acid sequence (SEQ ID NO:49) of the human G-protein coupled receptor, HGPRBMY8, comprising, or alternatively consisting of, one or more of the predicted polynucleotide polymorphic loci, in addition to, the encoded polypeptide polymorphic loci of the present invention for this particular protein.

On page 23, replace paragraph [0098] with the following paragraph:

[0098] In one of its embodiments, the present invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:2 as shown in Figure 1. The HGPRBMY8 polypeptide is 508 amino acids in length and shares amino acid sequence homology with the GPCR RE2. The HGPRBMY8 polypeptide (SEQ ID NO:2) shares

24.3 % identity and 33.6 % similarity with over 400 amino acids of the GPCR RE2 sequence, wherein “similar” amino acids are those which have the same/ similar physical properties and in many cases, the function is conserved with similar residues. For example, amino acids Lysine and Arginine are similar; while residues such as Proline and Cysteine, which do not share any physical properties, are considered dissimilar. The HGPRBMY8 polypeptide shares 28.01% identity and 38.33% similarity with the *Fugu rubripes* 5-Hydroxytryptamine 1a-Alpha Receptor (5H1A_FUGRU; SWISS-PROT Acc. No.:O42385); 25.3% identity and 37.23% similarity with the human 5-Hydroxytryptamine 1a-Alpha Receptor (5H1A_HUMAN; SWISS-PROT Acc. No.:P08908); 27.56% identity and 37.56% similarity with the *Mus musculus* 5-Hydroxytryptamine 1a-Alpha Receptor (5H1A_MOUSE; SWISS-PROT Acc. No.:Q64264, Q60956); 25.46% identity and 37.05% similarity with the *Lymnaea stagnalis* 5-hydroxytryptamine receptor (5HT_LYMST; SWISS-PROT Acc. No.:Q25414); 23.67% identity and 33.19% similarity with the *Bos taurus* Alpha-1A adrenergic receptor (A1AA_BOVIN; SWISS-PROT Acc. No.: P18130); 26.21% identity and 36.9% similarity with the *Canis familiaris* Alpha-1A adrenergic receptor (A1AA_CANFA; SWISS-PROT Acc. No.: O77621); 29.47% identity and 41.05% similarity with the human Alpha-1A adrenergic receptor (A1AA_HUMAN; SWISS-PROT Acc. No.: P35348); 31.65% identity and 42.29% similarity with the *Oryzias latipes* Alpha-1A adrenergic receptor (A1AA_ORYLA; SWISS-PROT Acc. No.:Q91175); 30% identity and 41.32% similarity with the *Oryctolagus cuniculus* Alpha-1A adrenergic receptor (A1AA_RABIT; SWISS-PROT Acc. No.: O02824); 24.82% identity and 34.43% similarity with the *Rattus norvegicus* Alpha-1A adrenergic receptor (A1AA_RAT; SWISS-PROT Acc. No.:P43140); 29.79% identity and 41.19% similarity with the human Alpha-1D adrenergic receptor (A1AD_HUMAN; SWISS-PROT Acc. No.: P25100); 29.2% identity and 40.57% similarity with the *Mus musculus* Alpha-1D adrenergic receptor (A1AD_MOUSE; SWISS-PROT Acc. No.:P97714, Q61619); 23.33% identity and 31.97% similarity with the *Gallus gallus* muscarinic acetylcholine receptor M4 (ACM4_CHICK; SWISS-PROT Acc. No.:P17200); 30.53% identity and 41.58% similarity with the *Mus musculus* Alpha-1A adrenergic receptor (O54913; SWISS-PROT Acc. No.:O54913); 29.47% identity and 41.05% similarity with the human

Alpha-1A adrenergic receptor isoform 4 (O60451; SWISS-PROT Acc. No.:O60451); 23.59% identity and 32.82% similarity with the human G-protein coupled receptor RE2 (O75963; SWISS-PROT Acc. No.:O75963); 23.99% identity and 31.81% similarity with the Branchiostoma lanceolatum dopamine D1/Beta receptor (O96716; SWISS-PROT Acc. No.:O96716); 29.21% identity and 40.79% similarity with the human Alpha 1C adrenergic receptor isoform 2 (Q13675; SWISS-PROT Acc. No.:Q13675); 24.87% identity and 34.52% similarity with the human Alpha 1C adrenergic receptor isoform 3 (Q13729; SWISS-PROT Acc. No.:Q13729); and 21.49% identity and 32.023% similarity with the *Caenorhabditis elegans* probable G protein coupled receptor F01E11.5 (YDBM_CAEL; SWISS-PROT Acc. No.:Q19084).

On page 38, replace paragraph [0147] with the following paragraph:

[0147] [0147] A wide variety of labels and conjugation techniques are known and employed by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding HGPRBMY8 polypeptide include oligo-labeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding HGPRBMY8 polypeptide, or any portions or fragments thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes *in vitro* by addition of an appropriate RNA polymerase, such as T7, T3, or SP(6) and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits (e.g., Amersham Pharmacia Biotech, Promega and U.S. Biochemical Corp.). Suitable reporter molecules or labels which may be used include radionucleotides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

On page 85, replace paragraph [0289] with the following paragraph:

[0289] Fluorescent emissions were detected using a Nikon-TE300 microscope equipped with an excitation filter (D405/10X-25), dichroic reflector (430DCLP), and a barrier filter for dual DAPI/FITC (510nm) to visually capture changes in Beta-Lactamase expression. The FACS Vantage SE is equipped with a Coherent Enterprise II Argon Laser and a Coherent 302C Krypton laser. In flow cytometry, UV excitation at 351-364 nm from the Argon Laser or violet excitation at 407 nm from the Krypton laser are used. The optical filters on the FACS Vantage SE are HQ460/50m and HQ535/40m bandpass separated by a 490 dichroic mirror.

On page 89, replace paragraph [0298] with the following paragraph:

[0298] The Aurora Beta-Lactamase technology provides a clear path for identifying agonists and antagonists of the HGPRBMY8 polypeptide. Cell lines that exhibit a range of constitutive coupling activity have been identified by sorting through HGPRBMY8 transfected cell lines using the FACS Vantage SE (see Figure 16). For example, cell lines have been sorted that have an intermediate level of orphan GPCR expression, which also correlates with an intermediate coupling response, using the LJI analyst. Such cell lines will provide the opportunity to screen, indirectly, for both agonists and ant[agonists] of HGPRBMY8 by looking for inhibitors that block the beta lactamase response, or agonists that increase the beta lactamase response. As described herein, modulating the expression level of beta lactamase directly correlates with the level of cleaved CCF2 substrate. For example, this screening paradigm has been shown to work for the identification of modulators of a known GPCR, 5HT₆, that couples through Adenylate Cyclase, in addition to, the identification of modulators of the 5HT_{2c} GPCR, that couples through changes in $[Ca^{2+}]_i$. The data shown below represent cell lines that have been engineered with the desired pattern of HGPRBMY8 expression to enable the identification of potent small molecule agonists and antagonists. HGPRBMY8 modulator screens may be carried out using a variety of high throughput methods known in the art, though preferably using the fully automated Aurora UHTSS system. The

untransfected CHO-NFAT/CRE cell line represents the relative background level of beta lactamase expression (Figure 16; panel a). Figure 16 describes several CHO-NFAT/CRE cell lines transfected with the pcDNA3.1 HygroTM / HGPRBMY8 mammalian expression vector isolated via FACS that had either intermediate or high beta lactamase expression levels of constitutive activation. Panel A shows untransfected CHO-NFAT/CRE cells prior to stimulation with 10 nM PMA, 1 μ M Thapsigargin, and 10 μ M Forskolin (- P/T/F). Panel B shows CHO-NFAT/CRE cells after stimulation with 10 nM PMA, 1 μ M Thapsigargin, and 10 μ M Forskolin (+ P/T/F). Panel C shows a representative orphan GPCR (oGPCR) transfected CHO-NFAT/CRE cells that have an intermediate level of beta lactamase expression. Panel D shows a representative orphan GPCR transfected CHO-NFAT/CRE that have a high level of beta lactamase expression. Following treatment with a cocktail of 10 nM PMA, 1 μ M Thapsigargin, and 10 μ M Forskolin (Figure 16; P/T/F; panel b), the cells fully activate the CRE-NFAT response element demonstrating the dynamic range of the assay. Panel C (Figure 16) represents an orphan transfected CHO-NFAT/CRE cell line that shows an intermediate level of beta lactamase expression post P/T/F stimulation, while panel D (Figure 16) represents a orphan transfected CHO-NFAT/CRE cell line that shows a high level of constitutive beta lactamase expression.

On page 92, replace paragraph [0307] with the following paragraph:

[0307] The Graph # of Table 1 corresponds to the tissue type position number of Figure 17. HGPRBMY8 (also known as GPCR84 or GPCR58) was found to have relatively low expression in the tumor cell lines assayed in the OCLP-1 (oncology cell line panel). HGPRBMY8 message appears to be especially scarce in breast tumor cell lines. The average HGPRBMY8 message [message] level in lung tumor cell lines is 2-3 fold lower than the average for other cell lines assayed.

On page 99, replace paragraph [0314] with the following paragraph:

[0314] Cyclic analogs were prepared from the crude linear products. The cysteine disulfide was formed using one of the following methods:

On page 100, replace paragraph [0321] with the following paragraph:

[0321] Briefly, using the isolated cDNA clone encoding the full-length HGPRBMY8 polypeptide sequence, appropriate primers of about 15-25 nucleotides derived from the desired 5' and 3' positions of SEQ ID NO:1 may be designed to PCR amplify, and subsequently clone, the intended N- and/or C-terminal deletion mutant. Such primers could comprise, for example, an initiation and stop codon for the 5' and 3' primer, respectively. Such primers may also comprise restriction sites to facilitate cloning of the deletion mutant post amplification. Moreover, the primers may comprise additional sequences, such as, for example, flag-tag sequences, [k]Kozac sequences, or other sequences discussed and/or referenced herein.

On page 117, replace paragraph [0360] with the following paragraph:

[0360] Low-density DNA chips (Affymetrix, CA) may be hybridized following the manufacturer's instructions. Briefly, the hybridization cocktail consisted of 3M TMACI, mM Tris pH 7.8, 0.01% Triton X-100, 100 mg/ml herring sperm DNA (Gibco BRL), 200 pM control biotin-labeled oligo. The processed PCR products are then denatured for 7 minutes at 100°C and then added to prewarmed (37°C) hybridization solution. The chips are hybridized overnight at 44°C. Chips are wa[i]shed in 1X SSPET and 6X SSPET followed by staining with 2 ug/ml SARPE and 0.5 mg/ml acetylated BSA in 200 ul of 6X SSPET for 8 minutes at room temperature. Chips are scanned using a Molecular Dynamics scanner.

On page 120, replace paragraph [0373] with the following paragraph:

[0373] The invention encompasses additional methods of determining the allelic frequency of the SNPs of the present invention. Such methods may be known in the art, some of which are described elsewhere herein.

On page 121, replace paragraph [0378] with the following paragraph:

[0378] There are two distinct types of analysis of target DNA for detecting polymorphisms. The first type of analysis, sometimes referred to as de novo characterization, is carried out to identify polymorphic sites not previously characterized (i.e., to identify new polymorphisms). This analysis compares target sequences in different individuals to identify points of variation, i.e., polymorphic sites. By analyzing groups of individuals representing the greatest ethnic diversity among humans and greatest breed and species variety in plants and animals, patterns characteristic of the most common alleles/haplotypes of the locus can be identified, and the frequencies of such alleles/haplotypes in the population can be determined. Additional allelic frequencies can be determined for subpopulations characterized by criteria such as geography, race, or gender. The de novo identification of polymorphisms of the invention is described in the Examples section.

On page 122, replace paragraph [0379] with the following paragraph:

[0379] The second type of analysis determines which form(s) of a characterized (known) polymorphism are present in individuals under test. Additional methods of analysis are known in the art or are described elsewhere herein.

On page 123, replace paragraph [0383] with the following paragraph:

[0383] An allele-specific primer hybridizes to a site on target DNA overlapping a polymorphism and only primes amplification of an allelic form to which the primer exhibits perfect complementarity. See Gibbs, Nucleic Acid Res. 17,2427-2448 (1989).

This primer is used in conjunction with a second primer which hybridizes at a distal site. Amplification proceeds from the two primers, resulting in a detectable product which indicates the particular allelic form is present. A control is usually performed with a second pair of primers, one of which shows a single base mismatch at the polymorphic site and the other of which exhibits perfect complementarity to a distal site. The single-base mismatch prevents amplification and no detectable product is formed. The method works best when the mismatch is included in the 3'-most position of the oligonucleotide aligned with the polymorphism because this position is most destabilizing elongation from the primer (see, e.g., WO 93/22456).